REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	Dec. 22, 1998	Final, 1 Jan 94 - 31 May 1997

4. TITLE AND SUBTITLE
Mechanisms of Microbially Influenced Corrosion of Stainless Steel and Copper in Marine Systems

5. FUNDING NUMBERS
Grant No. N00014-94-1-0441
PR Number: 96PR00665
AGO Code: N66020
Cage Code: 4B958

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Office of Naval Research, Code 1141SB
800 N. Quincy Street
Arlington, VA 22217

10. SPONSORING / MONITORING AGENCY REPORT NUMBER
Not Applicable

11. SUPPLEMENTARY NOTES
None

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Concentric Electrodes (CE) were used to initiate and monitor pitting corrosion of 304 stainless steel by sulfate-reducing bacteria. A current (approx. 10 uA/cm^2 at the anode) was applied between a small circular anode and a larger surrounding cathode, and microorganisms were introduced into the deaerated seawater medium over the next 48 hours. The applied current was then removed and maintenance of current was recorded. Bacterial metabolism was manipulated with metabolic inhibitors. Experiments were also conducted under aerobic conditions at lower initial current fluxes. The Scanning Vibrating Electrode Microscope (SVEM) was used to congruently map current density and the location microbial cells. The bacterium Oceanospirillum produces a copper-binding exopolymer which has been implicated in copper corrosion. Exopolymer was isolated and partially analyzed. The organism was grown in the presence or absence of Cu^{++}, and differences in polymer production were examined.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Corrosion, Biofilms, Sulfate-reducing bacteria, Electrochemical	4
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
unclassified	unclassified	unclassified	SAR

FINAL REPORT

Grant #: N00014-94-1-0441

PRINCIPAL INVESTIGATOR: Dr. D. C. White, R. J. Palmer, P. Angell, C.A. Flemming

INSTITUTION: Center for Environmental Biotechnology/Univ. Tennessee

GRANT TITLE: Mechanisms of Microbially Influenced Corrosion of Stainless Steel and Copper in Marine Systems

AWARD PERIOD: 1 January 1994 - 31 May 1997

OBJECTIVE: determine the mechanisms of microbially influenced corrosion of stainless steel and copper in marine systems.

APPROACH: Concentric Electrodes (CE) were used to initiate and monitor pitting corrosion of 304 stainless steel by sulfate-reducing bacteria. A current (approx. 10 $\mu A/cm^2$ at the anode) was applied between a small circular anode and a larger surrounding cathode, and microorganisms were introduced into the deaerated seawater medium over the next 48 hours. The applied current was then removed and maintenance of current was recorded. Bacterial metabolism was manipulated with metabolic inhibitors. Experiments were also conducted under aerobic conditions at lower initial current fluxes.

The Scanning Vibrating Electrode Microscope (SVEM) was used to congruently map current density and the location microbial cells.

The bacterium *Oceanospirillum* produces a copper-binding exopolymer which has been implicated in copper corrosion. Exopolymer was isolated and partially analyzed. The organism was grown in the presence or absence of Cu^{++}, and differences in polymer production were examined.

ACCOMPLISHMENTS: Experiments with 304-stainless-steel CEs indicated that only a mixed-species biofilm (*Desulfovibrio vulgaris* plus *Vibrio* sp.) could maintain a corrosion current. A monoculture of either organism could not maintain the current, and a co-culture maintained the current (3-5 $\mu A/cm^2$) for sometimes greater than 200 hours. Pits were infrequently visible on the anodes of several electrodes on which current was maintained. Inhibition of hydrogenases (periplasmic using cupric chloride, and cytoplasmic using carbon monoxide) after current had been maintained for 24 - 48 hours had no effect on the current, nor did killing the cells with glutaraldehyde. In some experiments, a slow decline of the maintained current to zero was seen. In these cases, *D. vulgaris* colonization was poor.

Two biofilm types were examined in the CE system. Type 1 biofilms contained *Desulfovibrio vulgaris* as the SRB, and either *Vibrio harveyi* or *V. natriegens* as the second bacterium. Current was maintained irrespective of the *Vibrio* sp. used, but *Vibrio* is required for current maintenance. Following current shutoff, a spike in the current was seen that was up to ten-fold greater than the original applied current. The spike decayed (generally over 2-3 hrs) to a level below that of the applied current. Spike duration was at least two hrs and no spike was

seen in sterile controls; charge buildup occurs through the biofilm. Corrosion products were formed and electrochemical data (EIS) indicate that corrosion took place. Type 2 biofilms had *Shewanella putrifaciens* (a facultatively anaerobic bacterium that uses iron, thiosulfate, and nitrate as terminal electron acceptors) as the SRB, and either *V. harveyi* or *V. natriegens* as the second bacterium. A high spike (95 $\mu A/cm^2$) which decayed gradually over a period of 25 hrs occurred in the *Shewanella/V. natriegens* biofilm. Spikes in Type 2 biofilms decayed more gradually thus resulting in higher current fluxes than in Type 1 biofilms. The species of *Vibrio* did not affect current maintenance. No pitting was observed, but corrosion products were formed and electrochemical data verified that corrosion occurred.

The coculture *D. vulgaris/V. harveyi* was assayed for its ability to corrode a more corrosion resistant steel (316L) under aerobic conditions. The *Vibrio* strain was introduced first to allow formation of microsites of reduced oxygen tension. 1 μA/cm2 current was applied (about 10-fold less than in anaerobic experiments) over a three-day period. No oxygen was introduced during this time (the culture was stagnant). The current spike after removal of the applied current lasted 12 minutes, and flow of fresh medium was initiated at this timepoint. A dramatic rise in the open circuit potential of the anode was seen after oxygen was reintroduced into the culture by the fresh medium. EIS measurements showed that pitting had occurred on the anode.

Oceanospirillum sp. was grown on a copper coupon; examination of the coupon with the SVEM revealed an area of relatively high cell density that mapped congruently with an area of high anodic current density. Using a stain that discriminates live cells from dead, cells in the area of higher current density were shown to be living whereas areas of lower current density contained primarily dead cells. A sterile control coupon had no anodic areas. Exopolymer isolated from *Oceanospirillum* biofilms was shown to consist of a single monomer (perhaps glucose) and the polymer contained bound cupric ions.

Oceanospirillum sp. was grown in continuous culture in the presence and in the absence of copper (0.15 g/L $CuSO_4$). Cell yields (wet wt) were 1.89 g (+ Cu) and 0.84 g (- Cu). Exopolymer was precipitated with isopropanol from concentrated/dialyzed culture supernatants. Polymer yield was 89 mg/g cells (+ Cu) and 357 mg/g cells (- Cu). The polymer from the + Cu culture had a distinct bluish coloration and analysis (ICP-AES) showed it to contain 0.39% (dry wt) Cu. The polymer obtained from the - Cu culture contained <0.01% Cu. Once precipitated, neither polymer sample was soluble in water.

A laser confocal microscope was acquired and was set up as a multi-user facility. The microscope was used to examine microbial colonization on a concentric electrode exposed to mixed cultures of SRB and Vibrio sp. No clear-cut heterogeneity of colonization was seen on this particular electrode, however no electrochemical data verifying corrosion was collected either.

CONCLUSIONS: Mixed-species biofilms are significantly more effective at creating or continuing corrosion of stainless steel in our laboratory model system. It seems that heterogeneity in species composition of the biofilm occurred dependent on location (anode vs. cathode). We were able to demonstrate microbially influenced corrosion of SS 316L in a

system not maintained anaerobically; i.e., conditions required for the growth of SRB and for damage to highly corrosion-resistant alloys can be set up in a laboratory situation analogous the "real-world" (where most microbial environments important to the Navy deals are aerobic or microaerophilic).

Corrosion of copper by a single-species biofilm likely proceeds through exopolymer-mediated processes. Interestingly, although copper did not inhibit the growth of these cells in the planktonic phase, it did reduce the production of exopolymer in cells exposed to copper.

Laser confocal microscopy is a powerful tool for direct examination of microbial biofilms in laboratory analogues of corrosion microcosms. It can be used to confirm or deny the existence of supposed biofilm heterogeneity in corrosion situations.

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